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Genome Note

Whole-genome sequencing of *Staphylococcus aureus* L401, a *mecA*-negative community-associated methicillin-resistant strain isolated from a healthy carrier

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ABSTRACT

Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a human pathogen of great concern owing to its antimicrobial resistance and virulence properties. Here we report the first draft genome sequence of a *mecA*-negative community-associated MRSA strain isolated from a healthy young Mexican paediatric carrier in order to reveal the genomic structure underlying the multidrug-resistant phenotype and to discover the virulence properties of this strain.
Methods: The draft genome sequence of *S. aureus* L401 was obtained using an Ion Torrent™ PGM platform. De novo assembled contigs were annotated, and antimicrobial resistance genes and virulence factors were identified using ResFinder and VirulenceFinder, respectively. In addition, a mutational survey of native *pbp*, *gdpP* and *yjbH* genes was performed. In silico multilocus sequence typing (MLST) and *spa* typing were also performed.
Results: S. aureus L401 has a genome size of 2 831 587 bp with 2799 protein-coding sequences. Various antimicrobial resistance genes conferring resistance to aminoglycosides, β-lactams, fluoroquinolones and macrolide–lincosamide–streptogramin B antimicrobials were found. Although both *mecA* and staphylococcal cassette chromosome *mec* (SCC*mec*) elements were absent, a missense mutation in PBP3 was identified. Moreover, genes encoding exfoliative toxin A, γ- and β-haemolysin, and several enterotoxins were also identified. *S. aureus* L401 belongs to ST109 and *spa* type t209.
Conclusion: The availability of this genome will allow an insight into *S. aureus* resistance and virulence determinants as well as its epidemiology, lineage, evolution and genomic features involved in the paediatric commensal carriage.

1. Introduction

Staphylococcus aureus is a Gram-positive bacterium belonging to the phylum Firmicutes that inhabits the human skin and mucosa and can be both a commensal and pathogenic organism. Methicillin-resistant *S. aureus* (MRSA) is considered a major nosocomial pathogen worldwide; however, recent studies have shown its dissemination to the community [1]. *S. aureus* causes a diverse range of diseases from superficial skin infections to osteomyelitis, endocarditis and bloodstream infections. Up to 90% of *S. aureus* strains causing infection in America are resistant to β-lactam drugs. These strains represent a risk of difficult-to-treat infections. Genomic study of the multiresistance phenotype and virulence properties of staphylococcal strains around the world is of great epidemiological interest. Here we present the draft genome sequence of *mecA*-negative MRSA strain L401, a multi-drug-resistant isolate recovered from a pharyngeal exudate from a healthy young child in a day-care centre in Mexico.

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2. Materials and methods

S. aureus L401 was identified by Gram staining, mannitol fermentation, DNase production, and catalase and coagulase reaction. A presumptive methicillin-resistant phenotype was assessed by culture on chromogenic agar for detection of MRSA strains. Antimicrobial susceptibility testing was performed by the agar disk diffusion method, whilst the minimum inhibitory concentration (MIC) of methicillin (concentration range 0.25–16 μg/mL) was determined by broth microdilution according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Genomic DNA was extracted using a ZR Fungal/Bacterial DNA Kit (Zymo Research Corp., Irvine, CA). Genome sequencing of strain L401 was performed using an Ion Torrent™ PGM platform (Life Technologies, Carlsbad, CA) with 200-bp chemistry on a 318 chip. Raw sequence data were assembled using SPAdes v.3.11.0. Gene prediction and annotation were performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v.4.2 and Rapid Annotation using Subsystem Technology (RAST), respectively. Antimicrobial resistance determinants and virulence factors were analysed using ResFinder v.3.0 and VirulenceFinder v.1.5, respectively. In addition, the nucleotide sequences of genes for native penicillin-binding protein (PBP) (*pbp1*, *pbp2*, *pbp3* and *pbp4*), *gdpP* and *yjbH* were compared with those for methicillin-susceptible *S. aureus* (MSSA) ATCC 25923, NCTC 8523 and MSSA 476 strains. Multilocus sequence typing (MLST) and *spa* typing were performed using MLST v.2.0 and spaTyper v.1.0 tools from the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/).

To obtain a picture of the genomic basis underlying the antimicrobial resistance and pathogenic potential of *S. aureus* strains around the world and their dissemination, a comparative analysis of the Mexican *S. aureus* L401 genome against *S. aureus* strains of different geographical origins was performed. Complete genome sequences of *S. aureus* strains Mu50, Newman, CA-347, T0131 and USA300_FPR3757 strains were used (Table 1).

3. Results

S. aureus L401 was resistant to ampicillin, cefotaxime, cefepime, cefuroxime, dicloxacillin, erythromycin and penicillin by the disk diffusion method, and an MIC of 8 μg/mL for methicillin was determined by broth microdilution. A total of 1 239 655 reads were assembled in 55 contigs with a predicted genome size of 2 831 587 bp and a GC content of 32.67%. A total of 2994 genes were identified, including 2799 protein-encoding genes, 79 RNA genes (16 rRNAs, 59 tRNAs and 4 ncRNAs) and 116 pseudogenes. According to RAST analysis, the closest genome is *S. aureus* NN50. In addition, RAST revealed 402 subsystems of which 39 features corresponded to resistance to antibiotics and toxic substances (teicoplanin, aminoglycosides, fluoroquinolones, fosfomycin, β-lactams, copper, cobalt, zinc, cadmium and arsenic), including one mercury resistance operon and two multidrug efflux pumps.

Genes conferring resistance to aminoglycosides, β-lactams and fluoroquinolones as well as a macrolide–lincosamide–streptogramin B (MLS_B) phenotype-related determinant were identified (Table 1). The virulence-associated genes *seo*, *sem*, *sei*, *seu*, *sen* and *seg* encoding enterotoxins O, M, I, U, N and G, respectively, were identified. Moreover, genes for β-haemolysin (*hbl*), exfoliative toxin A (*eta*), staphylokinase (*sak*), a staphylococcal complement inhibitor (*scn*), aureolysin (*aur*) and γ-haemolysin components (*hlgA–C*) were detected. Based on genetic variation of seven housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi* and *yqiL*) and the *spa* gene, *S. aureus* L401 was classified as ST109 and *spa* type t209.

Regarding comparative analysis of the resistance genes, all strains except *S. aureus* strain Newman showed a multidrug resistance feature (Table 1). The genomic resistance profile of L401 strain was similar to that of CA-347; however, strain CA-347 has duplications of *spc* and *erm(A)* genes and harbours the *mecA* determinant. No *mecA* gene or staphylococcal cassette chromosome *mec* (SCC*mec*) elements were identified in the genome of strain L401. Methicillin-resistant strains lacking *mec* have been reported and their resistance phenotype has been associated with β-lactamase hyperproduction and multiple mutations in native *pbp* genes, the *pbp4* promoter and/or in *gdpP* as well as *yjbH* genes leading to PBP4 overproduction [2–5]. Interestingly, in strain L401 a missense mutation in the *pbp3* gene, encoding native PBP3, was found resulting in a D195N substitution. Despite the presence of a β-lactamase-encoding gene (*blaZ*) and the amino acid substitution in PBP3, an alternative mechanism might be involved and needs to be explored further. In this work, the virulence factors contained in the compared genomes clustered depending on the pathogenic process in which they are implicated (Table 1).

All of the genomes showed similar virulence profiles, although the Panton–Valentine leukocidin (*lukF*-PV and *lukS*-PV) and toxic shock syndrome toxin-1 (*tst*) genes were only identified in USA300_FPR3757 and Mu50 genomes, respectively. Surprisingly, *S. aureus* L401 showed a unique trait by possessing the type A exfoliative toxin (ETA) that is able to cause staphylococcal scalded

Table 1
Comparative genome analysis of antimicrobial resistance and virulence genes in *Staphylococcus aureus* strains.

	Staphylococcus aureus strain					
	L401	Mu50	Newman	CA-347	T0131	USA300_FRP3757
Origin	Mexico	Japan	England	USA	China	USA
Accession no.	**PDEY00000000**	**NC_002758**	**NC_009641**	**NC_021554**	**NC_017347**	**NC_007793**
ST/*spa* type	ST109/t209	ST5/t002	ST254/t008	ST45/t004	ST239/t030	ST8/t008
Antimicrobial resistance genes						
Aminoglycosides	*spc*	*aac(6')–aph(2")*, *spc*, *aadD*, *spc*	–	*spc*, *aadD*, *spc*	*spc*, *spc*	–
β-Lactams	*blaZ*	*mecA*	–	*mecA*, *blaZ*	*blaZ*, *mecA*	*mecA*
Fluoroquinolones	*norA*	*norA*	*norA*	*norA*	*norA*	*norA*
MLS_B	*erm(A)*	*erm(A)*, *erm(A)*	–	*erm(A)*, *erm(A)*	*erm(A)*	*erm(C)*
Tetracyclines	–	*tet(M)*	–	–	*tet(M)*	*tet(K)*
Virulence factors						
Extracellular enzymes	*aur*	*splB*, *splA*, *aur*	*splE*, *splB*, *splA*, *aur*	*aur*	*splB*, *splA*, *aur*	*splE*, *splB*, *splA*, *aur*
Host immune response	*scn*, *sak*	*scn*, *sak*	*scn*, *sak*	*scn*, *sak*	*scn*, *sak*	*scn*, *sak*, *ACME*
Toxins	*eta*, *seo*, *sem*, *hlgA*, *sei*, *seu*, *hlgC*, *sen*, *hlgB*, *seg*, *hlb*	*lukD*, *lukE*, *seg*, *sen*, *seu*, *sei*, *sem*, *seo*, *sea/sep*, *hlb*, *sel*, *sec*, *tst*, *hlgA*, *hlgC*, *hlgB*	*lukD*, *lukE*, *sea/sep*, *hlb*, *hlgA*, *hlgC*, *hlgB*	*seg*, *sen*, *seu*, *sei*, *sem*, *seo*, *hlb*, *hlgA*, *hlgC*, *hlgB*	*lukD*, *lukE*, *sea/sep*, *hlb*, *hlgA*, *hlgC*, *hlgB*, *sek*, *seq*	*lukF*-PV, *lukS*-PV, *lukD*, *lukE*, *hlb*, *hlgA*, *hlgC*, *hlgB*, *sek*, *seq*

ST, sequence type; MLS_B, macrolide–lincosamide–streptogramin B.

skin syndrome. This finding reveals the pathogenic potential and dissemination risk of *S. aureus* L401 with such genomic weapons in children in the community.

4. Nucleotide sequence accession no

The Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number PDEY00000000. The version described in this paper is the first version (PDEY01000000).

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Competing interests

None declared.

Ethical approval

Not required.

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